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ON PROTEOLYTIC ENZYMES. II.

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SINCE the writing of the previous paper¹ on the vegetable proteolytic enzymes, an article by VERNON² has appeared in which he gives an account of his investigation of the distribution of erepsin in the animal body. Comparative tests were made of glycerin extracts from thirteen different tissues of the cat, eight of the rabbit, eight of the guinea pig, seven of the pigeon, eight of the frog, seven of the eel, six of the lobster, and three of the fresh water mussel. In every case an erepsin was found to be present. The comparison of the activity of the various extracts was effected by means of colorimetric estimations of the intensity of the biuret reaction after certain periods of digestion. The tissue extracts from the warm blooded animals were more active than those from the cold blooded animals; the extracts from the invertebrate tissues had a relatively slight action. In the warm blooded animals it is not the intestinal mucous membrane which is richest in erepsin, but the kidney in the cat, rabbit, and pigeon, and the pancreas in the case of the guinea pig.

It might be noted that VERNON gives no record of experiments made with the various extracts to determine whether or not they are incapable of digesting the proteids of the tissues in which the enzymes occur. His experiments, being all conducted with Witte peptone solutions, do not conclusively show that the enzymes whose activity was observed are incapable of acting on any proteids except albumoses and peptones.

In a former communication evidence was given to show that *Phaseolus vulgaris* contains in its seeds a fairly active proteolytic enzyme. No action of this protease on the proteids of the seed could be demonstrated. Its power to act on Witte peptone as a whole, and on the albumose fractions separated from it, could be readily shown. Moreover, as germination progressed the cotyledons at all stages contained this erepsin and at no period of germination could

¹ DEAN: BOT. GAZETTE 39:321. 1904.

² VERNON: Jour. Physiol. 32:33. 1904.

any evidence of a tryptic enzyme be obtained. Further studies have been made of this seed enzyme in the hope of throwing light on the processes occurring during germination.

FURTHER EXPERIMENTS WITH THE GERMINATING SEEDS OF *PHASEOLUS VULGARIS*

The following experiment was carried out to demonstrate the action of the seed ereptase of the bean on the proteoses to be obtained from phaseolin, the principal globulin of the seed. Phaseolin was prepared according to OSBORNE'S³ method from white medium field beans, the yield from two kilograms of beans amounting to something over 75^{gm}. A small amount of phaseolin was also obtained by the alcoholic precipitation of a part of the solution from which the phaseolin had separated on dialysis. This albumin gives a marked Adamkiewicz reaction.

By mixing a known weight of phaseolin with fifty times its weight of 2 per cent. sulphuric acid and heating for three and a half to four hours in a steam sterilizer, a slightly brown gelatinous fluid was obtained having the odor characteristic of acid proteid hydrolysis. This fluid was cooled, diluted with water, and the gelatinous anti-albumid removed by filtration. The filtrate from the anti-albumid was neutralized with barium hydroxide and the filtrate from the barium sulphate concentrated and treated with several volumes of alcohol. The mixture of proteoses obtained in this way was collected on a filter, washed with absolute alcohol and ether, and dried *in vacuo* over sulphuric acid.

To obtain an ereptase solution finely ground beans were extracted with water and the extract filtered through pulp filters until nearly clear. Ten cubic centimeters of this solution were placed in each of four dry test tubes and two of the portions boiled. Solutions of Witte peptone and phaseolin proteoses were prepared by dissolving 0.2^{gm} of each preparation in 25^{cc} of water. The test solutions were made up as follows:

1. 10^{cc} enzyme solution + 10^{cc} phaseolin proteose solution + 9 drops of toluol.
2. Same as no. 1, using boiled enzyme solution.
3. 10^{cc} enzyme solution + 10^{cc} Witte peptone solution + 9 drops of toluol.
4. Same as no. 3, using boiled enzyme solution.

³ OSBORNE: 17th Annual Report of the Conn. Agric. Experiment Sta., Part IV. 186. 1893.

The corked tubes were kept in the incubator for six days. At the expiration of that time each digestion was boiled, acidified with a couple of drops of acetic acid, and filtered. Ten cubic centimeters of each filtrate were removed to a clean tube, and, after the addition of 5^{cc} of 10 per cent. sodium hydroxide solution, dilute copper sulphate solution was added to the maximum biuret reaction. After standing for several minutes the intensity of the biuret reactions was compared. The comparison showed that a marked digestion of both proteose mixtures had taken place, the one from phaseolin being rather more vigorously attacked. The unboiled digestion with Witte peptone gave a tryptophan reaction with bromine water, the other unboiled digestion did not.

Several trials were made of the digestibility of the acid phaseolin prepared by heating phaseolin for a few minutes with dilute sulphuric acid. The results obtained indicated that the enzyme of the seed had, at the most, but a feeble action on this body.

Various observers have shown that the antiseptics used in enzyme experiments may exert an inhibiting effect on the action of the ferment. VINES demonstrated that in papain digestions where sodium fluoride is used the action goes but little beyond the stage where albumoses and peptones are formed; whereas with other antiseptics, hydrocyanic acid for example, a marked formation of amido-acids takes place as shown by the production of tryptophan. It might be argued that the toluol used throughout the experiments with Phaseolus had an inhibiting action on the enzyme, so that it was unable to attack the proteids of the seed and could only act on albumoses and peptones. The two following experiments were carried out to settle that point:

Experiment I.—Thirty-five grams of finely comminuted cotyledons from six-day old bean seedlings were extracted for one and a half hours with 175^{cc} of water. The extract was filtered nearly clear through pulp filters and then forced through a Pasteur-Chamberland filter into a sterile flask. Three portions of 25^{cc} each were measured with a sterile pipette into three small sterile flasks. To no. 1 nothing further was added; to no. 2 was added 0.07 gm of sterile citric acid, making the solution $\frac{N}{25}$ citric acid; the contents

of no. 3 were boiled. The cotton plugs in the flasks were replaced corks. After keeping the three flasks in the incubator for forty-one hours, 20^{cc} of tannic acid reagent (7 per cent. tannic acid in 2 per cent. acetic acid) were run into each. After standing a few moments the contents were filtered through dry filters and duplicates of 20^{cc} from each filtrate analyzed for nitrogen by the Kjeldahl method.

Analyses of filtrates from no. 1 gave 0.0036^{gm} N

Analyses of filtrates from no. 2 gave 0.0036^{gm} N

Analyses of filtrates from no. 3 gave 0.0033^{gm} N

Experiment II.—Another extract from cotyledons of six-day old seedlings was prepared by treating 28^{gm} of finely ground tissue with 140^{cc} of cold water for three hours. The extract was filtered as in the previous experiment and 25^{cc} of the filtrate removed into two sterile flasks. The contents of one flask were boiled and both were corked with sterile corks and kept in the incubator for three days. To test the ereptic activity of the bacteria-free filtrate 15^{cc} were placed in a test tube with 0.5^{gm} of Witte peptone and a little toluol. At the end of the digestion period the fluid in this tube was found to give a strong tryptophan reaction with bromine water, showing that the enzyme had not been held back by filtration through porcelain. The contents of the two flasks were treated as in the previous experiment:

Analyses of filtrate from unboiled digestion gave 0.0036^{gm} N.

Analyses of filtrate from boiled digestion gave 0.0030^{gm} N.

The differences between the boiled and unboiled digestions in these two experiments are so slight as to be within the limits of error of the method used and cannot be taken to show any hydrolysis of the proteid in the cotyledon extract.

PROTEOLYSIS DURING THE GERMINATION OF *PHASEOLUS VULGARIS*

We have every reason for believing that the germination of the bean is accompanied by a hydrolysis of the proteids therein contained, a hydrolysis which our experimental evidence leads us to conclude must be started, at least, by some other agency than an enzyme. That proteolysis does accompany germination of this seed is shown by the following experiments, where the amounts of

coaguable and non-coaguable nitrogen were determined in the seeds and in the cotyledons of young seedlings.

About 75^{gm} of beans were soaked in water and the cotyledons separated from the skins and embryonic plants. The cotyledons after being washed were dried at 60° C. and ground in a hand mill, yielding the preparation *A*. Another part of the same lot of beans was planted in the greenhouse and allowed to germinate for seven days. At the end of this time the somewhat shrunken cotyledons had been pushed above ground, had begun to turn green, and were separating to allow the plumules to push out. These cotyledons were removed from the plants, washed, and dried at 80° C. When dry they were ground in the mill, giving preparation *B*. Portions of both *A* and *B* were rubbed to the finest powder possible in the mortar and dried at 100° C. to constant weight. Duplicate portions of 1^{gm} each of *A* and *B* were then weighed into small beakers, 15^{cc} of water added, and, after bringing to a boil, 2 drops of 10 per cent. acetic acid were stirred in. After allowing the coagulated proteids to settle for a moment or two the contents of each beaker were transferred to a dry washed filter paper in a funnel held in a Kjeldahl digestion flask. The washing of the precipitates and their quantitative transfer to the filters was effected by the use of six portions of 5^{cc} each of boiling distilled water. After thoroughly draining, the filters with the contained precipitates were transferred to Kjeldahl flasks; the precipitates and filtrates were then analyzed for nitrogen with the following average results:

Total nitrogen in *A* = 0.0407^{gm} = 4.17 per cent.

Total nitrogen in *B* = 0.0396^{gm} = 3.96 per cent.

Percentage of nitrogen in *A* as coaguable proteids = 90.7 per cent.

Percentage of nitrogen in *B* as coaguable proteids = 61.4 per cent.

Percentage of nitrogen in *A* as soluble compounds = 9.3 per cent.

Percentage of nitrogen in *B* as soluble compounds = 38.6 per cent.

It is worth noting that in the germination of the bean the consumption of nitrogenous foods proceeds at practically the same rate as that of the non-nitrogenous materials stored there; as a consequence the percentages of nitrogen in the cotyledons in the various stages of germination are nearly constant. After the cotyledons have become emptied the percentage of nitrogen changes, since the cell walls of

the shriveled cotyledons make up so large a percentage of the dry weight.

The experiment described above shows that proteolysis actually does occur as a step in the process of the utilization of the stored proteid. It is of interest in this connection to see if this process occurs when the cotyledons are removed from the young seedlings and kept under sterile conditions. A series of preliminary tests showed that if the cotyledons of germinating beans are treated for twenty minutes with a 0.5 per cent. solution of mercuric chloride and then repeatedly washed with sterile water and kept in sterile tubes they will remain free from bacterial or fungal infection. The procedure kills the peripheral cells of the cotyledons, as shown by the fact that they do not turn green if kept in the light; whereas unsterilized cotyledons show a gradual development of chlorophyll under the same conditions of light and moisture. Comparative tests of the sterilized and unsterilized cotyledons showed that both contained ereptase, but that there was a noticeably smaller amount in the ones treated with mercuric chloride. It is probable that the poison not only killed the outer layer of cells, but also destroyed the enzymes contained in them.

Three-day old seedlings of *Phaseolus* were removed from the soil and the cotyledons separated. A quantity of these were washed, dried between 70° and 80° C., and ground in the mill, yielding preparation *C*. Ninety other perfectly sound cotyledons were selected, forty-five placed in each of two previously sterilized flasks, and kept covered with 0.5 per cent. mercuric chloride solution for twenty minutes. After pouring off the sublimate solution the cotyledons were washed five times with sterile water in quantities as great as that of the mercuric chloride solution used. In pouring off the last wash water sufficient was left in the flasks to keep the cotyledons moist. The flasks were closed with sterile corks and kept at room temperature—about 20° C. After three days one flask was opened and the contained cotyledons dried and ground, giving preparation *D*. The contents of the flask were judged to be sterile by the absence of any foreign growth and by the results of a transfer of a drop of the fluid from the bottom of the flask to a sterile agar tube. The second flask was kept unopened for five days longer and then the cotyledons in it were treated as the other portion, yielding preparation *E*.

Samples of preparations *C*, *D*, and *E* were finely ground, dried to constant weight and estimations of the coaguable and non-coaguable nitrogen were made in the same way as the similar determinations on *A* and *B*.

Total nitrogen in 1 ^{gm} of <i>C</i>	-	-	-	-	-	-	-	-	0.0426
Total nitrogen in 1 ^{gm} of <i>D</i>	-	-	-	-	-	-	-	-	0.0426
Total nitrogen in 1 ^{gm} of <i>E</i>	-	-	-	-	-	-	-	-	0.0432
Percentage of nitrogen in <i>C</i> as coaguable proteids						-	-	-	81.7
Percentage of nitrogen in <i>D</i> as coaguable proteids						-	-	-	88.0
Percentage of nitrogen in <i>E</i> as coaguable proteids						-	-	-	79.9
Percentage of non-proteid nitrogen in <i>C</i>	-	-			-	-	-	-	18.3
Percentage of non-proteid nitrogen in <i>D</i>			-	-	-	-	-	-	12.0
Percentage of non-proteid nitrogen in <i>E</i>	-	-			-	-	-	-	20.1

These results would indicate that under the conditions of the experiment there is no proteolysis occurring, although, as previously stated, the cotyledons still contain ereptase. A small increase in coaguable nitrogen was observed, followed by a rise to about the original amount. There seems to be no evident explanation for these small variations. Two facts may be noted: the oxygen supply to the living cells in the interior of the cotyledons was probably curtailed by the presence of the layers of dead cells on the surface; the correlation of the parts of the organism had been destroyed and the influence of that factor on the metabolic processes is not known. The results tend to emphasize the dependence of proteolysis in this seed upon the normal life of the organs.

DISTRIBUTION OF THE PROTEASES IN VARIOUS PARTS OF PLANTS OF *PHASEOLUS VULGARIS*

A large number of qualitative tests for proteolytic enzymes in various parts of the plants of *Phaseolus* were carried out, using the tryptophan reaction as an indication of proteolysis. It seems scarcely necessary to detail the methods and results in every instance. The experiments were conducted in a way analogous to those on the cotyledons in the various stages of germination, a full description of which was given in a former paper. In testing for a tryptic enzyme the tissues were sometimes allowed to autolyze without the addition of any further proteid, but in most cases edestin, phaseolin, or uncoagulated egg albumin was added. In searching for evidence of

tryptic action the filtrates obtained after coagulating the proteids of the digestion mixture were frequently tested with Millon's and the biruet reactions, as well as with the tryptophan reaction. The creptic activity was tested with Witte peptone. The antiseptic used was toluol. Tests were made of the following tissues:

1. The embryonic plants in the seeds (exclusive of the cotyledons).
2. The hypocotyls and plumules from three-day old seedlings; the hypocotyls were from 2 to 4^{cm} long.
3. A mixture of the hypocotyls and plumules from six-day old etiolated seedlings.
4. The roots, stems, and buds of seven-day old seedlings grown in the light were tested separately; the roots were copiously branched and the buds were just opening from between the cotyledons.
5. Whole young plants, exclusive of cotyledons, of ten-day old etiolated seedlings.
6. The leaves and buds, and the roots and stems, of eleven-day old seedlings grown in the greenhouse.
7. The leaves and buds, stems, and roots of thirteen-day old etiolated seedlings.
8. The same tissues of fourteen-day old non-etiolated plants.
9. The leaves and the stems of plants twenty-two days old.
10. The leaves and buds, the upper parts of the stems, the stems from the root crown to a centimeter above the scars of the cotyledons.
11. The roots, stems, and leaves of plants just coming into bloom.
12. The developing seeds and pods.

In every case a good tryptophan reaction was obtained when the tissues were allowed to autolyze in the presence of Witte peptone. In no case, with the questionable exception noted below, could evidence of the presence of an enzyme capable of attacking native proteids be obtained. The one exception was furnished by the tests conducted on young hypocotyls in which edestin was added to the mixture of minced tissues and water. In this case a very faint tryptophan reaction was obtained, sufficient to raise the question of the existence of a tryptase in these tissues. Accordingly the following experiment was carried out. Ten grams of minced hypocotyls (1 to 3^{cm} long) from four-day old bean seedlings were placed in each of two flasks and 25^{cc} of water added. One flask was heated for several minutes in the steam sterilizer and then cooled. To each flask was added 0.25^{cm} of phaseolin, 0.25^{cm} of phaselin, and 1^{cc} of toluol. The corked flasks were kept in the incubator for three days. After

the digestion the contents of each flask were strained through cotton gauze, and 20^{cc} of each fluid precipitated with an equal volume of tannic acid reagent. Duplicates of 15^{cc} each from both filtrates were analyzed for nitrogen.

Nitrogen in analyses of filtrates of unboiled digestion = 0.0036^{gm}

Nitrogen in analyses of filtrates of boiled digestion = 0.0035^{gm}

It is therefore evident that no enzyme capable of acting on the native proteids of the bean is present in the young hypocotyls.

COMPARATIVE CONTENT OF EREPTASE IN VARIOUS TISSUES OF *PHASEOLUS VULGARIS*

In making any study of the relative quantities of ereptase in different tissues it is necessary to have some basis of comparison. Manifestly the dry weight of vegetable tissues is not a very satisfactory standard, since in many cases the cell walls constitute the greater part of the dry weight. The ideal way would be to use the weight of protoplasm as a basis for comparison, but this is out of the question. The best substitute seemed to be the quantities of the nitrogen in the tissues. The first measurements of the amount of ereptic activity in the tissues were carried out in the following manner. Nitrogen determinations were made on the fresh tissues to be tested, and then portions of each tissue, of such a weight that each portion contained 0.02^{gm} of nitrogen, were weighed out into small tared flasks. Into each flask 10^{cc} of a 10 per cent. Witte peptone solution were measured and the total weight of the digestion made up to 25^{gm} by the addition of distilled water. After the addition of 1^{cc} of toluol to each digestion the flasks were tightly corked and kept in the incubator for twenty-four hours at 41° C., the contents of each flask being shaken twice during that period. At the close of the digestion each mixture was strained through dry cotton gauze, and 20^{cc} of each fluid mixed with 20^{cc} of tannic acid reagent. The precipitates were filtered off on dry filters and the filtrates analyzed for nitrogen in duplicates of 15^{cc} each. Minor changes were made in this procedure in the subsequent series of determination, so that the results obtained thereby were not strictly comparable with the first series; since it was impossible to test the same tissues again the results of the first, and less satisfactory series, are nevertheless given:

Tissue	Percentage of N	Weight of tissue used	N in 15 ^{cc} of filtrate from tannic acid precipitate
Leaves of mature plants.....	0.68	2.941 ^{gm}	0.0204 ^{gm}
Developing pods.....	0.39	5.130 "	0.0240 "
Developing seeds.....	0.95	2.105 "	0.0283 "

From such results as these it is impossible to tell how much of the nitrogen found in the filtrates from the tannic acid precipitation is due to products of the digestion of Witte peptone and how much is non-proteid nitrogen present in the tissues used. Moreover, Witte peptone is not completely precipitable by tannic acid, certain of the very soluble peptones not being thrown down by that reagent. In the remaining estimations of comparative ereptic power two digestions were made with each tissue, one a boiled control. As before, the quantity of tissue taken was that which should contain 0.02^{gm} of nitrogen. The digestion mixtures were made up to 30^{gm} instead of 25^{gm}, as this quantity was found to be more convenient. Moisture determinations were also made on each tissue so that comparisons of the dry weights could be made. The results of this study are given in the accompanying table.

The table shows that although the method is not absolutely exact, yet fairly good duplicates are obtained in different digestions with the same tissue. The digestion in a number of cases was vigorous, noticeably so with the roots where more than half of the Witte peptone in the digestion mixture was hydrolyzed. It should be said that the roots bore a few small tubercles which were removed so far as possible, yet the minute ones remaining may have had some influence on the results. It is suggestive to note that it is not the seeds and cotyledons which contain the most enzyme per unit of nitrogen, but rather it is those tissues where active metabolism is occurring. It is unfortunate that we are unable to use the weight of protoplasm as a basis for comparison. The use of the nitrogen content as a standard is well enough between the various active tissues, but when these are compared with tissues gorged with storage proteids it is evident that the storage tissue contains far less protoplasm to a given weight of nitrogen. The error which the presence of stored proteids introduces into our comparative results is evident in the case

Tissue used	Percent- age of nitro- gen	Weight of fresh tissue	Dry weight of the tissue used	N in 15 ^{cc} of filtrate from tannic acid ppt. unboiled	Calculated amount for whole un- boiled di- gestion	N in 15 ^{cc} of filtrate from tannic acid ppt. boiled	Calculated amount for whole di- gestion	Nitrogen converted by the di- gestion	Witte pep- tone corre- sponding to nitrogen converted ¹
Ungerminated seeds I.....	3.80	0.526gm	0.499gm	0.0141gm	0.0564gm	0.0108gm	0.0432gm	0.0132gm	0.0911gm
Cotyledons of 5-day seedlings.....	1.74	1.149	0.455	0.0156	0.0624	0.0117	0.0468	0.0156	0.1076
Cotyledons of 10-day seedlings.....	0.72	2.777	0.653	0.0213	0.0852	0.0120	0.0480	0.0372	0.2507
Ungerminated seeds II.....	3.80	0.526	0.499	0.0141	0.0564	0.0111	0.0444	0.0120	0.0828
Cotyledons of 3-day seedlings.....	1.71	1.170	0.404	0.0144	0.0576	0.0102	0.0408	0.0168	0.1159
Hypocotyls of 3-day seedlings.....	0.62	3.226	0.286	0.0174	0.0696	0.0114	0.0456	0.0240	0.1056
Roots—blossoming stage.....	0.31	6.452	0.807	0.0321	0.1284	0.0123	0.0492	0.0792	0.5465
Leaves—blossoming stage.....	0.72	2.777	0.404	0.0199	0.0796	0.0096	0.0384	0.0412	0.2843
Stems—blossoming stage.....	0.23	8.696	1.304	0.0240	0.0960	0.0126	0.0504	0.0456	0.3146
Ungerminated seeds III.....	3.80	5.26	.499	0.0138	0.0552	0.0111	0.0444	0.0108	0.0745
Stems—plants mixed age.....	0.23	8.696	1.304	0.0240	0.0960	0.0132	0.0528	0.0432	0.2981

¹ The Witte peptone used in these experiments was found to contain 14.5 per cent. of nitrogen; the figures in this column were accordingly obtained by multiplying the values of the preceding column by 6.9.

of the emptying cotyledons. The cotyledons of ten-day old seedlings contain much more ereptase than those of the three and five-day old ones. The ten-day cotyledons were green, shriveled, and probably contained no proteids except those of the protoplasm. As a consequence, the number of cotyledons needed to contain 0.02^{gm} of nitrogen was far larger, the number of cells used much greater, and the amount of protoplasm probably far in excess of the amount in the digestion with younger cotyledons and ungerminated seeds. It is doubtful if there is any increase of ereptase per cell during the process of germination; if that is the case, the amount of ereptase per unit of protoplasm contained in the seeds and young cotyledons is much more clearly indicated by the results obtained with the exhausted cotyledons than by those obtained with the storage tissues themselves. In many cases it is evident that there is no special quantity of ereptase formed in the germinating seeds for the purpose of digesting the reserve proteids. The unexpected activity of the root tissue is probably connected with some processes, as yet unknown, which occur there and which are associated with the nitrogenous metabolism of those organs. VERNON obtained just such a suggestive result with the kidneys of several warm blooded animals; it is probable that there are processes occurring in that organ which are now unknown, but which involve a rapid nitrogenous metabolism.

CONCLUSIONS.

The results of this study have shown that, as was to be expected, the proteids of the seeds of *Phaseolus vulgaris* undergo proteolysis during germination as a preliminary to the transportation of the nitrogen and its utilization in the formation of new organs. There are three ways in which this process might be carried out: by the action of a tryptic enzyme; by the combined action of the protoplasm and of an enzyme which is, by itself, incapable of carrying out the whole process; or by the action of the protoplasm alone. The results of a study of the proteolytic enzymes of the resting and germinating bean show that there is no enzyme present which is able to digest the proteids of the seed. There is present, however, an enzyme of the ereptase group which is capable of digesting the proteoses resulting from the partial hydrolysis of the seed proteids. It may be,

therefore, that the protoplasm of the cells starts the process of proteid decomposition, and carries it to some stage at which the ereptase takes up the work and completes the process. That the protoplasm has some hand in the process is made evident by the fact that killing the protoplasm is sufficient to stop the proteolysis, although the means taken are those which do not inhibit the action of enzymes. Moreover, when the cells of the cotyledons are placed under abnormal conditions, the hydrolysis of the proteids ceases. It is, of course, possible that the complete hydrolysis is carried out by the protoplasm and that the ereptase has no share in it. There is something to be said for either view. It might be pointed out that the ereptic activity as shown by the tissues of the cotyledons when acting on Witte peptone is quite sufficient to accomplish the ereptic part of the complete proteolysis. Moreover, the very fact that an enzyme is present which is capable of doing part of the work of cleavage would lead one to infer that it had some part to play in the process. On the other hand, it has been shown that the cotyledons contain relatively less ereptase than the ordinary vegetative organs, and that there is no increase, or at the most a very slight one, in the enzyme content during germination.

The investigation of the other organs of the plant shows that they all contain the ereptase in somewhat varying quantities, the roots containing the most per unit of nitrogen. There is no evident explanation for these quantitative variations, nor for the function of the enzyme in the metabolism of the cells.

We have good reasons for believing that the life of every active cell is intimately bound up with a round of chemical changes of which one part consists in a cleavage of the protoplasmic proteids. It is quite conceivable that the means by which the cleavage is effected may vary in different cells; in some the whole process may be carried out by the protoplasm itself, unassisted by any of its enzymatic tools; in other cells the protoplasm may start the process and split off proteoses from itself which are subsequently attacked by an ereptic enzyme and broken down to the amido-acids, hexon bases etc.; in still other cases it may be that the complete hydrolysis is carried out by an enzyme or combination of enzymes. The bridging of the gap between widely different forms by a series of small variations

is a characteristic of the morphology of living organisms. That same sort of bridging is no less characteristic of their physiological processes. For example, we find all stages of dependence upon oxygen, from plants which are killed by its absence to those which are killed by its presence. It is not inconceivable that there exists an analogous range of differences in the means which various plants take to accomplish the cleavage of their metabolic proteids.

At any rate it has been shown by the researches of various investigators that enzymes are present in animal and vegetable tissues of both the tryptic and ereptic type. There has not yet been found a tissue which has been definitely shown to contain no proteolytic enzymes whatever, and which must therefore complete its processes of proteolysis by the activity of its protoplasm. Our knowledge is too limited to allow us to say that such tissues do not exist; that point must be left for future investigations.

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